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ENDONEURIAL BLOOD FLOW IN RAT SCIATIC NERVE DURING DEVELOPMENT

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SUMMARY

1. Endoneurial blood flow (EBF) in the sciatic nerve of rats aged 2–12 weeks was studied using microelectrode H_2 polarography.

2. EBF is highest in 2-week-old rats and progressively declines during development. Mean arterial pressure (MAP) is low at 2 weeks of age, gradually increases through the next 4 weeks, and is relatively constant thereafter. The decrease in EBF, in spite of an increase in MAP, occurs because the endoneurial vascular resistance is increasing faster than the MAP.

3. The higher EBF in younger rats is not due to the smaller diameter of their nerves. Sural and tibial nerves of 12-week-old rats, with diameters comparable to that of the sciatic nerve of a 3-week-old rat, have EBFs similar to that of the sciatic nerve of a 12-week-old rat.

4. There was no compelling evidence of autoregulation of EBF in 3-week-old rats over a MAP range from -40 to $+30$ mmHg of the normal value.

5. The increase of nerve vascular resistance with maturation is probably due to a decrease in capillary density and, to a lesser extent, to an increase in plasma viscosity and haematocrit.

6. The higher EBF in immature rats is likely to be a developmentally adaptive mechanism which permits greater blood–nerve exchange of material to accommodate the greater metabolic needs of rapidly elongating and myelinating axons and proliferating Schwann cells.

INTRODUCTION

The rate of blood flow in adult mammalian sciatic nerve has been studied with H_2 microelectrode polarography (Smith, Kobrine & Rizzoli, 1977; Low & Tuck, 1984), laser–Doppler flowmetry (Rundquist, Smith, Michel, Ask, Oberg & Rapoport, 1985; Takeuchi & Low, 1987), and rapidly diffusing radiotracers (Rundquist *et al.* 1985; Sladky, Greenberg & Brown, 1985; Sugimoto, Monafo & Eliasson, 1986). The salient characteristics which emerge from these studies are: (1) even though endoneurial and

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sheath (epineurium and perineurium) blood flows are interconnected, the former can be measured separately; (2) endoneurial flow consists of a larger nutritive component and a smaller capacitative component; (3) there is no evidence of autoregulation of endoneurial blood flow (EBF); and (4) segmental endoneurial vasculature is nourished by axial vessels from adjoining segments and by transperineurial radial vessels arising from the sheath vasculature.

Brain, heart and kidney depend on a regular supply of blood-borne substrates in order that oxidative metabolism meets their energy requirements and, thus, have a relatively tight coupling between blood flow and metabolism. They also have blood flow rates greater than 35 ml min^{-1} (100 g)$^{-1}$. On the other hand, nerves are relatively resistant to ischaemia (Poole, 1956; Seneviratne & Peiris, 1968; Low, Schmelzer & Ward, 1986), and EBF is 10–15 ml min^{-1} (100 g)$^{-1}$. This suggests that nerves do not depend on a moment-to-moment supply of vascularly derived energy substrates and the coupling between metabolism and blood flow is not as tight as that in the brain. Thus, nerves appear to cope with acute increases in metabolic needs with, at most, a doubling of blood flow and by relying on endoneurial reserves of high energy phosphates.

Therefore, it is of interest to examine the mechanisms by which immature rat nerves, with higher rates of metabolism and oxygen consumption (Low *et al.* 1986), maintain an adequate vascular perfusion of the endoneurium, especially during this period of lower mean arterial pressure (MAP). Furthermore, since tissues with higher metabolic rates normally exhibit autoregulation, it seemed pertinent to investigate whether EBF in sciatic nerves of 3-week-old rats is autoregulated.

Abstracts of this investigation have been presented (Kihara, Weerasuriya & Low, 1989; Kihara, Ward, Weerasuriya & Low, 1990).

METHODS

Using the microelectrode H_2 clearance technique, EBF was measured at rest in sciatic nerves of rats ranging in age from 2 to 12 weeks. Also recorded were their body weight, MAP during the experiment, and blood pH, P_{CO_2}, and P_{O_2} at the end of the experiment. To ascertain the autoregulatory capacity of the immature endoneurial vasculature, EBF was also studied in 3-week-old rats whose blood pressure was varied by altering their blood volumes.

Male Sprague–Dawley rats (Harlan Laboratories, St Paul, MN, USA) were used in these experiments. The rat pups used for the 2 and 3 week time points were received with either their mother or a lactating female. All animals were kept for a few days in the animal care facility before being used in the experiments. They were housed in plastic cages with wood shavings on the bottom, and had unlimited access to drinking water and food.

Surgical preparation. The details of the procedure have been previously described (Low & Tuck, 1984). Briefly, rats were anaesthetized with Inactin (Byk Gulden, Konstanz, Germany; 100 mg kg^{-1}, I.P.), and their rectal temperature was maintained between 36·5 and 37·0 °C with a thermostatically driven infra-red lamp positioned above the animal. Muscle paralysis was induced with tubocurarine (15–20 U kg^{-1}, I.A. or I.P.). Tracheostomized rats were ventilated using a rodent ventilator (Harvard Apparatus, Millis, MA, USA). Blood gases were maintained within the physiological range. Polyethylene catheters were placed in the left common carotid artery and left jugular vein. Blood pressure was monitored and blood gases were sampled through the arterial catheter. MAP showed no spontaneous fluctuations, and it did not change in response to manipulation of tissues under sufficiently deep anaesthesia. When such fluctuations appeared, usually toward the end of an experiment, additional increments of Inactin (8–12 mg kg^{-1}) were given by slow I.A. injection. To abolish muscle twitches, additional tubocurarine (3–5 mg kg^{-1}, I.A.) were administered slowly when necessary.

H_2 polarography. A length of the mid-thigh sciatic nerve, not exceeding 1 cm, was exposed and a pool was formed with the surrounding muscle and skin. This pool was filled with mineral oil maintained at 33·5 ± 0·1 °C with a servo-controlled infra-red lamp. One end of a polyethylene tube, filled with 2 M-KCl in 3% agar, was inserted into the abdominal subcutaneous tissue. The other end was connected to the reference terminal of a current-sensitive amplifier (Chemical Microsensor, Diamond Electrotech, Ann Arbor, MI, USA). Microelectrodes (tip diameter 2–5 μm) were constructed as previously described (Low & Tuck, 1984). An electrode, chosen for its linear response to varying concentrations of H_2 in water, was inserted into the sciatic nerve through the perineurium. Using a calibrated micromanipulator, the electrode was positioned in the centre of the nerve by inserting it a distance equal to half the diameter. The free end of the electrode was connected to the current-sensitive amplifier and polarized positively to 0·25 V. When a stable baseline current had been established, 10% H_2 was added to the inspired gas mixture and the N_2 concentration was reduced to maintain a constant physiological arterial P_{O_2}. After the resulting increase in current had reached a plateau, H_2 was turned off and N_2 concentration was readjusted. The H_2 polarographic current then decreased exponentially toward the baseline value. The output from the amplifier was transmitted through an analog to a digital converter (Coulbourn L25-08, Coulbourn Instruments, Lehigh Valley, PA, USA) for simultaneous display (Labtech Notebook, Laboratory Technologies Corporation, Wilmington, MA, USA) and storage (Lotus 1-2-3, Lotus Development Corporation, Cambridge, MA, USA). A monoexponential or biexponential equation was fitted to the data using a non-linear curve-fitting program based on the Marquardt–Levenburg algorithm (Labtech Notebook).

Autoregulation. Using 3-week-old rats, autoregulation was examined by measuring EBF at four or five different MAPs in each animal. MAP was altered by either exsanguination or reperfusion through the venous catheter.

Influence of nerve diameter. To examine the effect of nerve diameter on EBF, blood flow was measured in the sural and tibial nerves of 12-week-old rats. The diameters of these nerves are comparable to that of the sciatic nerve of a 3-week-old rat.

RESULTS

The MAP and body weight of rats during the first 12 weeks of development are shown in Fig. 1. There were six or seven animals in each age group. Body weight increased continuously during this period, but MAP, after increasing during the first 6 weeks, remained relatively constant over the next 6 weeks.

Figure 2 depicts the pattern of change of EBF during development. The changes in endoneurial vascular resistance (calculated from the EBF and MAP) during this same period are also shown in Fig. 2. In spite of an increase in MAP, EBF decreases because of an even faster rate of increase of endoneurial vascular resistance. A few of the nerves (16%) exhibited a biexponential H_2 clearance curve, suggestive of a significant capacitative blood flow component. There were no age-related trends in the incidence of these endoneurial shunt pathways.

EBF in sural and tibial nerves of 12-week-old rats was not significantly different from that in the 12-week-old rat sciatic nerve. On the other hand, they were both significantly ($P < 0·02$) lower than that of the 3-week-old rat sciatic nerve. The data presented above, together with the blood P_{CO_2}, P_{O_2} and pH are summarized in Table 1.

The effect of varying the MAP on EBF is shown in Fig. 3. The data are from four experiments in 3-week-old rats. There was a direct relationship between the MAP and EBF. Deviations of the MAP larger than that shown in Fig. 3 were compensated for by cardiovascular regulatory mechanisms during the course of EBF measurement. In order to examine the autoregulatory capacity, the changes in EBF were

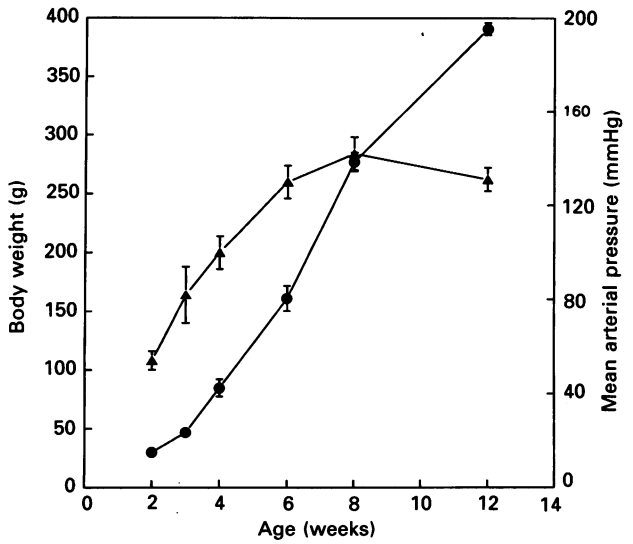

Fig. 1. Mean arterial pressure (▲) and body weight (●) of rats during development. In this and following figures and table, data are presented as means ± S.E.M.

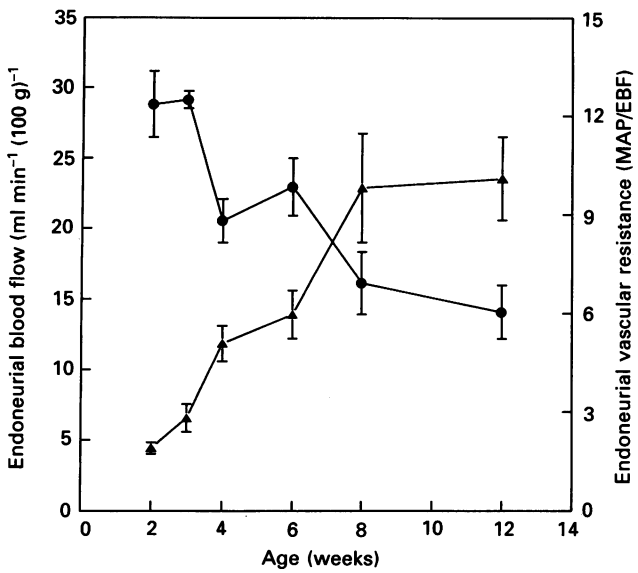

Fig. 2. Endoneurial vascular resistance (▲) and blood flow (●) in rat sciatic nerve during development. Data are presented as means ± S.E.M.

expressed as percentages of EBF before blood volumes were altered. These results are presented in Fig. 4. A straight line, with a correlation coefficient of 0·98 ($P < 0·01$), can be fitted to the data by linear regression although a linear increment was absent

TABLE 1. Endoneurial blood flow, mean arterial pressure and vascular resistance of nerve and body weight and blood gas parameters in rat during development

Age (weeks)	Wt (g)	EBF (ml min^{-1} $(100\ g)^{-1}$)	MAP (mmHg)	EVR (MAP/EBF)	P_{O_2} (mmHg)	P_{CO_2} (mmHg)	pH	EPV (ul g^{-1})
2	29·8 ± 1·7	28·8 ± 2·4	54 ± 4	1·9 ± 0·2	123·7 ± 12·0	16·7 ± 1·7	7·56 ± 0·06	3·92 ± 0·53
(sciatic)	(6)	(6)	(6)	(6)	(6)	(6)	(6)	
3	46·8 ± 3·5	29·2 ± 0·6	82 ± 12	2·8 ± 0·4	133·3 ± 9·2	14·8 ± 1·0	7·84 ± 0·09	3·97 ± 0·50
	(6)	(6)	(6)	(6)	(4)	(4)	(4)	
4	84·7 ± 7·3	20·5 ± 1·5	100 ± 7	5·1 ± 0·5	120·2 ± 7·9	24·7 ± 2·7	7·69 ± 0·6	2·24 ± 0·09
	(7)	(7)	(7)	(7)	(7)	(7)	(7)	
6	161·0 ± 10·8	23·0 ± 2·0	130 ± 7	5·9 ± 0·7	139·2 ± 20·7	31·1 ± 1·6	7·61 ± 0·02	2·58 ± 0·32
	(6)	(6)	(6)	(6)	(4)	(4)	(4)	
8	277·4 ± 7·9	16·1 ± 2·2	142 ± 7	9·8 ± 1·7	136·7 ± 8·3	27·7 ± 1·3	7·69 ± 0·03	1·50 ± 0·14
	(6)	(6)	(6)	(6)	(6)	(6)	(6)	
12	390·6 ± 5·1	14·1 ± 1·9	131 ± 5	10·1 ± 1·3	129·6 ± 4·2	22·6 ± 2·1	7·71 ± 0·04	1·48 ± 0·10*
	(6)	(6)	(6)	(6)	(6)	(6)	(6)	
12	383·7 ± 14·9	16·3 ± 1·3	145 ± 10	9·1 ± 0·8	141·4 ± 9·7	24·6 ± 1·4	7·61 ± 0·04	—
(sural)	(6)	(6)	(6)	(6)	(6)	(6)	(6)	
12	364·1 ± 6·9	12·7 ± 0·9	155 ± 7	12·6 ± 1·2	134·1 ± 3·9	26·6 ± 0·9	7·63 ± 0·04	—
(tibial)	(6)	(6)	(6)	(6)	(6)	(6)	(6)	

EBF, endoneurial blood flow; EPV, endoneurial plasma volume; MAP, mean arterial pressure; EVR, endoneurial vascular resistance. Numbers in parentheses indicate the number of observations.

* Measured at 13 weeks; data from Weerasuriya *et al.* (1990).

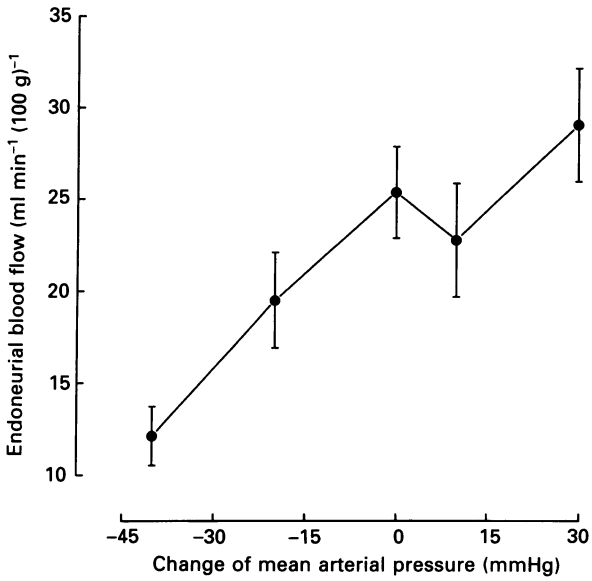

Fig. 3. Endoneurial blood flow in rat sciatic nerve as a function of mean arterial pressure in 3-week-old rats.

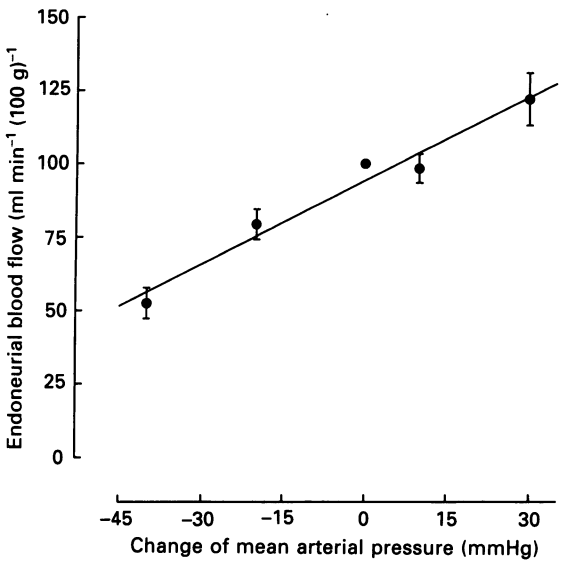

Fig. 4. Endoneurial blood flow (EBF) in rat sciatic nerve as a function of mean arterial pressure (MAP) in 3-week-old rats. Values on the ordinate are given as a percentage of EBF recorded before MAP was experimentally altered. The linear regression fitted to the data is $y = 98 + 0{\cdot}95x$ with $r = 0{\cdot}983$ ($P < 0{\cdot}01$).

between 0 and +15 mmHg. This indicates that blood flow in the sciatic nerve endoneurium of a 3-week-old rat is not convincingly autoregulated.

DISCUSSION

Significant conclusions of this study include the facts that the EBF of 2- to 3-week-old rats is about 3 times that of the adult rat and that the endoneurial vascular resistance of rat sciatic nerve increases during the first 12 weeks of development. This increase, which is greater than the simultaneous increase in MAP, is associated with an age-dependent decrease in EBF. The higher EBF of the infant rats could not be attributed to the smaller diameter of their nerves, and there was no evidence that autoregulation plays a role in maintaining the higher vascular perfusion in these nerves.

The use of microelectrode H_2 polarography to assess vascular perfusion in the rat sciatic nerve endoneurium has been extensively discussed and reviewed (Low & Tuck, 1984; McManis, Yao & Low, 1986; Day, Lagerlund & Low, 1989). An aspect of the present study that has not been reviewed is the influence of fascicle diameter on blood flow measurements. The absence of significant differences between the EBFs in sciatic, sural and tibial nerves of 12-week-old rats indicates that the decrease in sciatic nerve EBF with increasing age is an age-dependent phenomenon which is not related to fascicle size. The incidence of capacitative blood flow (16%) observed in this study is comparable to the frequency reported earlier (Low & Tuck, 1984; Day *et al.* 1989). This suggests that, during development, the basic endoneurial vascular architecture, at least in terms of the relative proportions of shunt pathways and exchange microvessels, is conserved.

The increase in endoneurial vascular resistance during development is due to changes in the rheological properties of blood and density and characteristics of microvessels in the endoneurium. The increases in plasma protein concentration and haematocrit during growth (Hitzig, 1963) would elevate the viscosity of blood and hence the vascular resistance. The slight increase in red cell rigidity with age would also contribute to the increase in vascular resistance. On the other hand, physiological measurements of endoneurial vascular space with ^{131}I-labelled albumin between 2 and 13 weeks of age indicate an approximately 2·7-fold decrease in vascular volume per gram of endoneurium (Weerasuriya, Curran & Poduslo, 1990). If it could be assumed that endoneurial capillary length and radius did not change during development, then the above factors would be responsible for the increase in endoneurial vascular resistance reported in this study. However, because resistance is inversely proportional to the fourth power of the radius, even slight decreases in microvascular radius with age would significantly increase endoneurial vascular resistance.

The adult sciatic nerve endoneurium does not significantly autoregulate its vascular perfusion (Low & Tuck, 1984; Rundquist *et al.* 1985; Takeuchi & Low, 1987). Since tissues such as brain and kidney, with higher blood flow rates, autoregulate their vascular perfusion, it seemed reasonable to expect that 2- and 3-week-old rats, with their higher EBF, would exhibit some degree of autoregulation. However, the results described here suggest that the morphometric characteristics of

the endoneurial vasculature and physical properties of the blood, and not significant physiological autoregulatory mechanisms, are responsible for the higher EBF in infant rats. Given the relatively robust resistance of nerves to ischaemia, it is unlikely that a brief (5–10 min) period of reduced blood flow and attendant ischaemia would have deleterious effects on the nerve. Furthermore, if infant nerves are less vulnerable to anoxia than adult nerves (comparable with the difference in anoxic susceptibility between infant and adult brains (Johanson, 1989)), then they would be even more resistant to ischaemia and could withstand longer periods of reduced blood flow. It may be for this reason that injured juvenile mammalian nerves have better regenerative capacity (Black & Lasek, 1979), in addition to their greater growth and myelinating potential (Webster & Favilla, 1984).

Cerebral blood flow is autoregulated in newborn sheep (Purves & James, 1959) and dog (Hernandez, Brennan & Bowman, 1980), but not in rat (Purin & Syutkina, 1977). The presence of autoregulation in the former two species is thought to reflect a more advanced stage of central nervous system development in these two species (Gregoire, Gjedde, Plum & Duffy, 1978). For example, rat pups are born with their eyes shut whereas they are open at birth in sheep and dogs (Johanson, 1989). On the other hand, morphological tracer studies suggest that the blood–nerve interface develops more uniformly during the first few neonatal weeks (Kristensson & Olsson, 1971; Malmgren & Brink, 1975; Towfighi & Gonatas, 1977) among the various mammalian species.

If the endoneurium relied upon diffusional exchange across the perineurium for its metabolic needs and clearance of catabolic waste products, then smaller nerves, with a higher surface area-to-volume ratio, should have a smaller EBF. The absence of a difference between the adult EBF of larger (sciatic) and smaller (tibial and sural) nerves (Table 1) is consistent with transperineurial exchange playing a relatively minor role in the nourishment of the endoneurium. This is further supported by the relative impermeability (Weerasuriya, Rapoport & Taylor, 1979) and lack of morphological polarity (Olsson & Reese, 1971) of the perineurium, and the absence of transperineurial transport of ions (Weerasuriya, Rapoport & Taylor, 1980) and amino acids (Wadhwani, Smith & Rapoport, 1989). Nevertheless, the differential susceptibility of centrifascicular and perifascicular fibres under certain conditions (McManis & Low, 1988) would indicate that the endoneurium is not a well-mixed homogeneous compartment.

The developmental changes in EBF and vascular resistance and, by implication, those of capillary density and surface area (per unit volume of tissue) differ from those described as occurring during the first few weeks of postnatal cerebral development (Bar, 1980; Kreisman, Olson, Horne & Holtzman, 1989). Density of neuronal and glial processes, volume of mitochondria, concentration and activities of mitochondrial enzymes, O_2 consumption, and glucose metabolism increase sharply during the first 4 weeks of postnatal development (Ford, 1973; Miller, 1981; Cremer, 1982; Holtzman & Olson, 1983). These changes are accompanied by an increase in cerebral capillary density and blood flow (Bar, 1980; Kreisman *et al.* 1989) demonstrating the ontogenetic aspects of the relatively tight coupling between cerebral metabolism and blood flow. Maturational changes in nerve also reveal a qualitatively similar coupling although the age-dependent trend is towards decreases

in O_2 consumption, high energy phosphate expenditure, endoneurial vascular volume, and EBF (Low *et al.* 1986; Weerasuriya *et al.* 1990).

The elevated EBF, reported here, coupled with the higher permeability coefficient–surface area product of the infant blood–nerve interface to small (sodium) and large (albumin) solutes (Weerasuriya, 1989; Weerasuriya *et al.* 1990), are likely to be developmentally adaptive mechanisms. These provide greater blood–nerve exchange of materials to accommodate the greater metabolic needs of rapidly elongating and myelinating axons and proliferating Schwann cells.

In summary, the developmental decrement in EBF parallels the reduction in nerve O_2 consumption and metabolism, and an increase in endoneurial vascular resistance is the major factor responsible for this reduction in EBF. The endoneurial homeostatic mechanisms that co-ordinate and regulate the changes in endoneurial energy supply and demand have not been elucidated. Identification of the relevant factors and their modes of operation would not only provide a better understanding of how the constancy of the endoneurial microenvironment is maintained but also aid in targeting therapeutic measures to treat peripheral neuropathies and nerve trauma.

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